

Cystatin-C, Renal Function, and Incidence of Hip Fracture in Postmenopausal Women

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OBJECTIVES: To evaluate the association between chronic kidney disease and incident hip fracture using serum cystatin-C as a biomarker of renal function calculated without reference to muscle mass.

DESIGN: Case-control study nested within a prospective study.

SETTING: The Women's Health Initiative Observational Study conducted at 40 U.S. clinical centers.

PARTICIPANTS: From 93,676 women aged 50 to 79 followed for an average of 7 years, 397 incident hip fracture cases and 397 matched controls were studied.

MEASUREMENTS: Cystatin-C levels were measured on baseline serum using a particle-enhanced immunonephelometric assay. Estimated glomerular filtration rates (eGFR_{cys-c}) were calculated using a validated equation and categorized into three groups (≥ 90.0 mL/min per 1.73 m², 60.0–89.9 mL/min per 1.73 m², and < 60.0 mL/min per 1.73 m² indicating chronic kidney disease Stages 3 to 4).

RESULTS: The odds ratio (OR) for hip fracture was 2.50 (95% confidence interval (CI) = 1.32–4.72) for eGFR_{cys-c} less than 60 mL/min per 1.73 m² compared with Stages 0 to 1, after adjustment for body mass, parental hip fracture, smoking, alcohol consumption, and physical function. No association was observed for eGFR_{cys-c} of 60 to 90 mL/min per 1.73 m² (OR = 1.04, 95% CI = 0.66–1.64). Additional adjustment for poor health status, hemoglobin, serum 25-hydroxy vitamin D, and bone metabolism markers did not affect these associations. Adjustment for plasma homocysteine reduced the OR for eGFR_{cys-c} less than 60 mL/min per 1.73 m² to 1.83 (95% CI = 0.93–3.61).

CONCLUSION: Women with eGFR_{cys-c} levels less than 60 mL/min per 1.73 m² have a substantially greater risk of hip fracture. Effects of renal function on homocysteine levels may partially mediate, or accompany, this association. *J Am Geriatr Soc* 56:1434–1441, 2008.

Key words: chronic kidney disease; hip fracture; cystatin-C; renal function

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Severe kidney disease appears to increase the risk of hip fracture.^{1,2} Chronic kidney disease (CKD) is common, but mild and moderate stages are frequently clinically unrecognized and therefore understudied. Two prior prospective studies support an association between moderate renal insufficiency as measured using serum creatinine estimated glomerular filtration rates (eGFR) or cystatin-C levels and greater hip fracture risk in older women. In both studies, associations were lower after full multivariate adjustment,^{3,4} although renal function biomarkers such as eGFR from serum creatinine are dependent on age and muscle mass, two major determinants of fracture risk. A method of estimating renal function that is not calculated using muscle mass or body size has potential to better define the risk of fractures due to emerging kidney disease.

Cystatin-C is a 122-amino acid, 13-kDa protein originating broadly from nucleated cells that is filtered by the kidney and completely metabolized by the proximal tubule. Serum cystatin-C levels are reportedly independent of age and lean tissue mass and potentially superior to serum creatinine in detecting mild to moderate renal impairment. The results of a large case-control study nested within the prospective Women's Health Initiative Observational Study (WHI-OS) designed to determine whether cystatin-C levels are related to risk of hip fracture in postmenopausal women, independent of other hip fracture risk factors and in the absence of hormone therapy and osteoporosis medications, are reported here. Potential explanatory factors for an association between renal function and osteoporosis and hip fracture are explored.

METHODS

Study Group

The WHI-OS is a prospective cohort study that enrolled 93,676 women aged 50 to 79 from 1994 to 1998 at 40 clinical centers throughout the United States. Study methods have been described in detail elsewhere.⁵ Briefly, women were eligible if they were postmenopausal, unlikely to move or die within 3 years, not enrolled in the WHI Clinical Trial, and not currently participating in any other clinical trial. At baseline, women completed screening and enrollment questionnaires by interview and self-report, a physical examination, and blood specimen collection. Human subjects review committees at each participating institution reviewed and approved the study.

Follow-Up and Outcome Ascertainment

Women were sent questionnaires annually to report the occurrence of any hospitalization and a wide variety of outcomes including clinical fractures of any type. Follow-up time ranged from 0.7 to 9.3 years per participant as of August 2004, with a median duration of 7.13 years. At that time, 3.7% of WHI OS participants had withdrawn or were lost to follow-up, and 5.3% had died. Trained physicians verified hip fractures by reviewing radiological, magnetic resonance imaging, or operative reports at each clinical center, and blinded central adjudicators confirmed.⁶ Hip fractures with a possible or confirmed pathological cause (from malignancy, infection, or focal bone lesion) were excluded.

Nested Case-Control Study Design

The present study is a case-control study nested within the prospective design of the WHI-OS using incident hip fracture cases identified through August 2004. Participants were excluded if they had a prior history of hip fracture at baseline or were taking osteoporosis treatments (bisphosphonates, calcitonins, parathyroid hormone). Because endogenous hormone levels were also under investigation, women taking estrogen up to 1 year before enrollment or currently taking androgens (anabolic steroids, dehydroepiandrosterone, testosterone), selective estrogen receptor modulators, or antiestrogens were also excluded. Women without sufficient serum or with unknown ethnicity were also excluded, leaving a final study

group of 39,795 eligible participants. From this group, 404 incident cases of hip fracture were identified. One control per case was selected with individual matching according to age at screening (± 1 year), race or ethnicity, and date of blood draw (± 120 days). Cystatin-C levels were obtained in 397 matched pairs.

Baseline Clinical Variables

All covariates were ascertained at baseline. Clinic interviewers recorded current use of prescription medications, including thiazide diuretics and corticosteroids, at the first screening visit by directly inspecting medicine containers. Prescription names were entered into the WHI database, which assigned drug codes using Medispan software.

Dietary supplements, including calcium preparations, taken at least twice weekly for the prior 2 weeks were entered directly from medicine containers as described above. Dietary intake of calcium was measured using a semi-quantitative food-frequency questionnaire.⁷ Total calcium intake was defined as the sum of calcium from diet, supplements, and medications.

Baseline questionnaires ascertained information on race or ethnicity, age at menopause, personal history of fracture after age 55, treated diabetes mellitus, myocardial infarction, coronary revascularization or stroke, current and past smoking, parental history of hip fracture, and self-rated health status. Alcohol consumption was estimated using questionnaire items as servings per week. Physical activity was classified based on frequency and duration of four speeds of walking and mild, moderate, and strenuous activities in the prior week. Kilocalories of energy expended in a week on leisure-time activity was calculated (metabolic equivalent score = kcal h/wk per kg).⁸ Physical function was measured using the 10-item Rand-36 Physical Function Scale, which includes items measuring whether health now limits physical function in moderate to vigorous activities; strength to lift, carry, stoop, or bend; ability to climb stairs; ability to walk various distances without difficulty; and self-care.⁹ Frailty was defined as a score of 3 or more based on the sum of poor physical function (2 points), low physical activity, exhaustion, and weight loss as described previously using a measure validated in the WHI-OS.¹⁰ Weight was measured to the nearest 0.1 kg on a balance beam scale with the participant dressed in indoor clothing without shoes. Height was measured to the nearest 0.1 cm using a wall-mounted stadiometer. Body mass index (BMI) was calculated as weight (kg)/height (m^2).

Laboratory Procedures

Laboratory personnel were blinded to case-control status for all measurements. Serum cystatin-C levels were measured using the Dade Behring BN-II nephelometer and Dade Behring reagents (GMI Inc., Ramsey, MN) using a particle-enhanced immunonephelometric assay at Medical Research Laboratories International (Highland Heights, KY). The assay has a sensitivity of 0.02 mg/L and an inter-assay coefficient of variation of 5.7%. The measurement range is 0.25 to 7.9 mg/L, with a reference range for ostensibly healthy people aged 1 to 78 of 0.53 to 0.93 mg/L. Estimated glomerular filtration rate ($eGFR_{cys-c}$) was calculated using the formula $76.7 \times \text{cystatin-C}^{-1.18}$, which has

been validated in large populations using urinary clearance of ^{125}I -iothalamate (51Cr-ethylenediaminetetraacetic acid).¹¹

Several laboratory biomarkers were investigated as potential mechanisms for an association between renal function and hip fracture. Renal function influences serum total homocysteine, which has been associated with risk of hip fracture.^{12–15} Levels were measured in fasting samples using a high-performance liquid chromatography assay at the same laboratory. The coefficient of variation was 7.3% to 7.6%, with a range of 5 to 15 $\mu\text{mol/L}$. As part of the baseline screening process, hemoglobin levels were measured in local laboratories using standard clinical procedures for complete blood count. Serum 25-hydroxy vitamin D (25(OH)D) was quantified according to radioimmunoassay using reagents from Diasorin (Stillwater, MN). C-terminal telopeptide of Type I collagen (CTX) and aminoterminal procollagen extension propeptide (PINP) were measured according to immunoassay at Synarc (Lyon, France).

Statistical Methods

Baseline characteristics of hip fracture cases and matched controls were compared, with corresponding *P*-values calculated from chi-square tests for categorical variables and *t*-tests for continuous variables. To further assess the potential for confounding, baseline characteristics were compared across quartiles of cystatin-C levels in control participants. Associations between cystatin-C levels and incident hip fracture were assessed in conditional logistic regression models retaining the matched case-control design (age, race or ethnicity, blood draw date). Associations were first examined without any additional adjustment and then with adjustment for BMI (continuous), parental history of hip fracture, smoking, alcohol use, and RAND-36 physical function score (>90 , ≤ 90). Covariates were selected for inclusion in the full multivariate model based on their association with incident hip fracture in the initial univariate analysis and their correlation with cystatin-C levels. Correlations between cystatin-C and other biomarker levels were assessed using Pearson correlation coefficients (*r*).

Cystatin-C levels were evaluated as a continuous variable and also across quartile categories defined based on distribution in the control subjects. Using the $\text{eGFR}_{\text{cys-c}}$ formula, cystatin-C levels were categorized and analyzed per mL/min per 1.73 m^2 in three groups (>90 , $60\text{--}90$, and <60).¹⁶ Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated from the conditional logistic regression models per standard deviation difference for continuous level of cystatin-C and in comparison with the best renal function group in the categorical models. To investigate mechanisms through which cystatin-C might be associated with hip fracture, a base model was constructed with adjustment for BMI, parental history of hip fracture, smoking, and alcohol, and then the following variables were added one at a time to determine their effect on the cystatin-C ORs: markers for deteriorating health status (poor physical function, frailty score, number of chronic conditions, hemoglobin level), homocysteine, 25(OH)D, and bone turnover markers (CTX and PINP).

RESULTS

The mean age of cases and controls was 71, and 95% were Caucasian (Table 1). Cases had lower BMI and were more likely to be current smokers, use corticosteroids, have a history of stroke, exercise less, and have lower physical function scores.

Cystatin-C levels were weakly correlated with age ($r = 0.22$) and BMI ($r = 0.19$) (Table 2). There was an association between physical function and cystatin-C, with 47% of women in the lowest quartile reporting high

Table 1. Baseline Characteristics of Hip Fracture Cases and Controls

Characteristic	Control Case		P-Value
	n (%)		
Ethnicity			1.00
White	380 (95.0)	380 (95.0)	
Black	10 (2.5)	10 (2.5)	
Hispanic	2 (0.5)	2 (0.5)	
American Indian	3 (0.8)	3 (0.8)	
Asian or Pacific Islander	5 (1.3)	5 (1.3)	
Age at screening			1.00
50–59	25 (6.3)	25 (6.3)	
60–69	107 (26.8)	107 (26.8)	
70–79	268 (67.0)	268 (67.0)	
Body mass index, kg/m^2			.001
<25.0	144 (36.1)	193 (48.6)	
25.0–29.0	150 (37.6)	127 (32.0)	
≥ 30.0	105 (26.3)	77 (19.4)	
History of fracture on or after age 55	82 (20.5)	96 (24.0)	.23
Parent had broken hip	64 (16.0)	80 (20.0)	.14
Hormone replacement therapy usage			.80
Never used	302 (75.5)	305 (76.3)	
Past user	98 (24.5)	95 (23.8)	
Oral daily corticosteroid use	3 (0.8)	14 (3.5)	.007
RAND 36—Physical Functioning score >90	117 (30.1)	84 (21.8)	.009
General health			.06
Excellent to very good	220 (56.0)	194 (48.9)	
Good	131 (33.3)	142 (35.8)	
Fair to poor	42 (10.7)	61 (15.4)	
Treated diabetes mellitus (pills or injections)	19 (4.8)	24 (6.0)	.43
Alcohol use			.61
Nondrinker	70 (17.6)	58 (14.6)	
Past drinker	80 (20.2)	89 (22.4)	
<7 drinks per week	205 (51.6)	212 (53.3)	
≥ 7 drinks per week	42 (10.6)	39 (9.8)	
Smoking			.00
Never smoked	215 (54.3)	214 (54.3)	
Past smoker	171 (43.2)	144 (36.6)	
Current smoker	10 (2.5)	36 (9.1)	
History of myocardial infarction	14 (3.5)	22 (5.5)	.17
History of stroke	8 (2.0)	18 (4.5)	.05

Table 2. Baseline Characteristics According to Quartiles of Cystatin-C in the Control Group

Characteristic	Quartile of Serum Cystatin-C*				P-Value
	1	2	3	4	
	n (%)				
Ethnicity					
White	97 (93.3)	90 (91.8)	92 (95.8)	98 (99.0)	.008
Black	0 (0.0)	5 (5.1)	4 (4.2)	1 (1.0)	
Hispanic	1 (1.0)	1 (1.0)	0 (0.0)	0 (0.0)	
American Indian	3 (2.9)	0 (0.0)	0 (0.0)	0 (0.0)	
Asian or Pacific Islander	3 (2.9)	2 (2.0)	0 (0.0)	0 (0.0)	
Age at screening					
50–59	12 (11.5)	7 (7.1)	4 (4.2)	2 (2.0)	< .001
60–69	46 (44.2)	22 (22.5)	21 (21.9)	16 (16.2)	
70–79	46 (44.2)	69 (70.4)	71 (74.0)	81 (81.8)	
Body mass index, kg/m²					
18.5–24.9	53 (51.5)	43 (43.9)	29 (30.2)	19 (19.2)	< .001
25.0–29.9	33 (32.0)	38 (38.8)	36 (37.5)	42 (42.4)	
≥30.0	17 (16.5)	17 (17.4)	31 (32.3)	38 (38.4)	
Parent had broken hip	16 (15.4)	18 (18.4)	14 (14.6)	15 (15.2)	.89
Hormone replacement therapy usage					
Never used	81 (77.9)	71 (72.5)	72 (75.0)	76 (76.8)	.82
Past user	23 (22.1)	27 (27.6)	24 (25.0)	23 (23.2)	
Oral daily corticosteroid use	0 (0.0)	0 (0.0)	2 (2.1)	1 (1.0)	.28
General health (self-report)					
Excellent to very good	62 (60.8)	57 (59.4)	54 (56.8)	46 (47.4)	.41
Good	28 (27.5)	32 (33.3)	30 (31.6)	40 (41.2)	
Fair to poor	12 (11.8)	7 (7.3)	11 (11.6)	11 (11.3)	
Treated diabetes mellitus (pills or injections)	5 (4.8)	4 (4.1)	4 (4.2)	5 (5.1)	.99
Alcohol use					
Nondrinker	11 (10.7)	21 (21.4)	17 (17.7)	20 (20.6)	.01
Past drinker	19 (18.5)	13 (13.3)	26 (27.1)	20 (20.6)	
<7 drinks/wk	53 (51.5)	52 (53.1)	48 (50.0)	52 (53.6)	
≥7 drinks/wk	20 (19.4)	12 (12.2)	5 (5.2)	5 (5.2)	
RAND 36 Physical Functioning score >90	48 (47.1)	33 (34.4)	21 (22.8)	15 (15.6)	< .001
Thiazides and thiazide-like diuretic use	3 (2.9)	5 (5.1)	7 (7.3)	8 (8.1)	.39
Smoking					
Never smoked	55 (53.4)	47 (48.0)	52 (55.3)	59 (60.2)	.75
Past smoker	46 (44.7)	48 (49.0)	39 (41.5)	37 (37.8)	
Current smoker	2 (1.9)	3 (3.1)	3 (3.2)	2 (2.0)	

* Quartiles defined based on the distribution of cystatin-C levels in the control group. Cases are omitted from the table. P-values from chi-square test or Fisher exact test.

function, versus 16% of women in the highest quartile ($r = -0.24$). Frequent alcohol consumption was more common in women with lower cystatin-C levels. Levels of other fracture risk factors varied little across quartiles of cystatin-C.

The unadjusted OR for incident hip fracture comparing the highest and lowest quartiles of cystatin-C levels was 1.51 (95% CI = 0.98–2.33). A greater increase in risk occurred after adjustment for BMI (OR = 2.14, 95% CI = 1.33–3.43), and this association persisted after additional adjustment for parental hip fracture, smoking, alcohol consumption, and physical function score. Highly significant linear trends between cystatin-C level considered as a continuous variable in the multivariate models

and incident fracture were observed, although most of the risk appeared to be concentrated in the upper quartile (Table 3).

Conversion of cystatin-C levels into eGFR_{cys-c} categories classified 133 women with levels greater than 90 mL/min per 1.73 m², 517 with levels between 60 and 90 mL/min per 1.73 m², and 144 women with levels less than 60 mL/min per 1.73 m². The latter group included 138 women with CKD Stage 3 (eGFR_{cys-c} 30–59 mL/min per 1.73 m²) and six with CKD Stage 4 (eGFR_{cys-c} 15–29 mL/min per 1.73 m²). No women had Stage 5 CKD (eGFR_{cys-c} <15 mL/min per 1.73 m²). Table 4 presents associations between these eGFR_{cys-c} categories and risk of hip fracture without adjustment, adjusted for BMI alone, and then in

Table 3. Relationship Between Cystatin-C Levels and Risk of Hip Fracture

Cystatin-C*	Unadjusted	Adjusted for BMI	Multivariate Adjusted†
	Odds Ratio (95% Confidence Interval)		
Per mg/L increase	2.08 (1.15–3.77)	3.01 (1.54–5.87)	2.92 (1.38–6.21)
Per standard deviation (0.27 mg/L) increase	1.22 (1.04–1.43)	1.35 (1.12–1.61)	1.34 (1.09–1.64)
<i>P</i> for linear trend	.02	< .001	.005
Number of missing pairs (total = 400)	6	10	32
Quartile (mg/L cutpoint)			
1 (0.58–0.90)	1	1	1
2 (0.91–1.00)	0.91 (0.59–1.39)	1.08 (0.69–1.68)	1.07 (0.66–1.76)
3 (1.01–1.14)	1.14 (0.74–1.75)	1.41 (0.89–2.22)	1.42 (0.86–2.34)
4 (1.15–3.68)	1.51 (0.98–2.33)	2.14 (1.33–3.43)	2.07 (1.21–3.55)

* Hip fracture case and control selection matched on age, ethnicity, and blood draw date. Quartile cutpoints defined based on distribution in controls.

† Multivariate adjustment included body mass index (BMI), parental history of hip fracture, smoking, alcohol use, and RAND 36 physical functioning score.

full multivariate models. The adjusted OR relating eGFR_{cys-c} less than 60 mL/min per 1.73 m² to hip fracture was 2.50 (95% CI = 1.32–4.72), but no association was seen between the group with eGFR_{cys-c} 60 to 90 mL/min per 1.73 m² (OR = 1.04, 95% CI = 0.66–1.64) and that with eGFR_{cys-c} greater than 90 (Table 4). Additional adjustment for physical activity did not alter these results, nor did adjustment for thiazide diuretic use, loop diuretic use, years since menopause, or total calcium intake. In separate conditional logistic models for hip fracture subtypes, the OR for eGFR_{cys-c} less than 60 mL/min per 1.73 m² was high for femoral neck fracture (OR = 3.89, 95% CI = 1.71–8.83; 227 matched pairs) but not for trochanteric fracture (OR = 0.98, 95% CI = 0.30–3.20, 135 matched pairs), although the numbers of case–control pairs according to subtype were small, CIs overlapped, and the difference in ORs was not statistically significant.

ORs for eGFR_{cys-c} categories were similar regardless of which variable was used to account for overall poor health status (physical functioning, frailty score, number of chronic conditions, hemoglobin) (Table 4). In addition, adjustment for bone biomarkers (CTX, PINP) did not affect ORs. The observed correlation between cystatin-C and plasma homocysteine was 0.45. Adjustment for plasma homocysteine reduced the OR for eGFR_{cys-c} less than 60 mL/min per 1.73 m² to 1.83 (95% CI = 0.93–3.61). In contrast, adjustment for serum 25(OH)D levels ($r = -0.10$ with cystatin-C) somewhat strengthened the association between eGFR_{cys-c} less than 60 mL/min per 1.73 m² and hip fracture (OR = 2.95, 95% CI = 1.55–5.62) (Table 4).

Because associations between cystatin-C and hip fracture risk became stronger after adjustment for BMI, additional analyses were conducted to elucidate whether associations were consistent across BMI stratum and to

Table 4. Risk of Hip Fracture According to Baseline Estimated Glomerular Filtration Rate (eGFR) Categories as Defined According to Serum Cystatin-C

Variables Included in the Multivariate Model	<i>r</i> *	eGFR _{cys-c} Category in mL/min/1.73 m ²			<i>P</i> -Trend
		>90	60–<90	<60	
		Odds Ratio (95% Confidence Interval)			
Unadjusted		1.0	0.82 (0.56–1.20)	1.51 (0.92–2.47)	.10
Adjusted for BMI	0.19	1.0	0.99 (0.66–1.48)	2.27 (1.31–3.94)	.003
Base analysis†		1.0	1.10 (0.70–1.71)	2.64 (1.41–4.97)	.003
Base analysis†+RAND 36 physical functioning >90	–0.24	1.0	1.04 (0.66–1.64)	2.50 (1.32–4.72)	.005
Base analysis†+frailty score	0.19	1.0	1.05 (0.67–1.64)	2.52 (1.33–4.77)	.005
Base analysis†+number of chronic conditions	0.18	1.0	1.05 (0.67–1.65)	2.49 (1.32–4.70)	.005
Base analysis†+plasma homocysteine	0.45	1.0	0.96 (0.60–1.51)	1.83 (0.93–3.61)	.105
Base analysis†+25hydroxyvitamin D	–0.10	1.0	1.20 (0.76–1.90)	2.95 (1.55–5.62)	<.001
Base analysis†+hemoglobin	–0.11	1.0	1.08 (0.69–1.70)	2.74 (1.44–5.19)	.002
Base analysis†+C-terminal telopeptide of Type I collagen	0.20	1.0	1.11 (0.71–1.73)	2.51 (1.33–4.74)	.005
Base analysis†+aminoterminal procollagen extension propeptide	0.14	1.0	1.10 (0.71–1.73)	2.68 (1.41–5.08)	.003

* Pearson correlation coefficient between cystatin-C and the additional risk factor.

† Matched on age, ethnicity, blood draw date, controlled for body mass index (BMI), parental history of hip fracture, smoking, and alcohol use. Number of missing case–control pairs ranges from 32 to 39 in these analyses out of 397 total case–control pairs.

test for interaction between the two variables. Using conditional logistic regression retaining the matched design, whether ORs differed for cystatin-C levels analyzed as a continuous variable and by $eGFR_{cys-c}$ categories in women with high and low BMI defined by the median cutpoint in controls (26.91 kg/m²) was tested. ORs indicated greater risk of hip fracture in both BMI strata, with somewhat stronger associations in overweight women in both analyses. For cystatin-C as a continuous variable, the OR in overweight women was 1.64 per standard deviation increase (95% CI = 1.21–2.23), compared with an OR of 1.12 in thinner women (95% CI = 0.90–1.39; *P*-value for interaction = .03). ORs comparing low (<60 mL/min per 1.73 m²) and high (>60 mL/min per 1.73 m²) $eGFR_{cys-c}$ categories were 1.99 for thinner women (95% CI = 0.98–4.03) and 2.41 (95% CI = 2.03–2.85) for overweight women (*P* value for interaction = .68).

DISCUSSION

This prospective, nested, case-control investigation of cystatin-C levels shows a strong, independent association between $eGFR_{cys-c}$ levels less than 60 mL/min per 1.73 m² and greater risk of hip fracture in postmenopausal women. Women with impaired renal function had 2.5 times the risk of hip fracture independent of well-established fracture risk factors including age, BMI, and physical function.

Studies relating renal function to risk of hip and other osteoporotic fractures are few in number. In women requiring kidney dialysis, hip fracture rates were found to be 17 times as high as in the general U.S. population.² Cross-sectional studies have shown associations between CKD defined using serum creatinine and self-reported history of hip and other fractures in the United States and Germany, but these studies were unable to determine which condition occurred first.^{17,18} Two recent epidemiological studies of older adults not selected on the basis of clinical kidney disease reported hazard ratios between serum creatinine $eGFR$ levels less than 60 mL/min per 1.73 m² and hip fracture ranging from 1.4 to 1.9.^{3,4} These associations were not statistically significant after full multivariate adjustment in either study, including calcaneal bone density in one study,³ but nonetheless supported an association between impaired renal function and hip fracture. Both previous studies had fewer than half the number of hip fractures investigated in this report (<200 vs 400). Cystatin-C levels were significantly associated with greater risk of hip fracture in women in one prior study (adjusted hazard ratio = 1.7 for the fourth vs first quartiles, 95% CI = 1.01–2.73).⁴ Especially strong associations with trochanteric versus femoral neck fractures were also seen in one study,³ whereas the opposite pattern was observed in the present study. Measures of health status and frailty did not explain the divergent patterns of association in either study.

A major question to resolve is whether the association between renal function and hip fracture reflects abnormalities in bone metabolism associated with renal osteodystrophy. CKD could lead to a greater risk of fractures in association with secondary hyperparathyroidism, osteomalacia, iron or aluminum bone disease, adynamic bone disease, or osteoporosis. Although clinical studies show that patients with severe kidney disease have lower bone density,

especially at cortical sites, epidemiological studies of renal function and bone density are inconsistent. Although some cross-sectional studies have shown differences in bone density between people with CKD and comparison subjects,^{19–21} another study did not find evidence of an independent association.²² Recent prospective analyses have shown no association with bone loss in women²² or a significant association with serum creatinine and bone loss only if analyzed using the Cockcroft-Gault equation.²⁰ Bone quality could be compromised with renal insufficiency even if density is not low. Adjustment for markers of bone resorption and formation did not alter the ORs for cystatin-C $eGFR_{cys-c}$ categories, suggesting mechanisms independent of bone turnover or more-complicated pathways than could be detected with the present methods.

In addition to abnormal physiology that could directly impair bone quality, CKD is often associated with poor health status, leading to frailty, falls, and ultimately fracture. However, ORs for CKD in this study were not lower after adjustment for physical function, frailty, number of chronic conditions, or anemia. Adjustment for serum 25(OH)D levels also did not reduce the OR for cystatin-C–determined CKD stage here or in a previous study.³ Cystatin-C levels may predict hip fracture, because the rate of decline in renal function is a strong indicator of biological aging independent of chronological age and clinically manifest disease. Biomarkers are lacking for exploring this hypothesis.

Recent experimental studies show that poor renal function as measured according to cystatin-C is an important determinant of homocysteine levels in older adults regardless of vitamin B₁₂ and folate status, which likely explains the correlation of 0.45 between the two biomarkers in this study.¹² Previous studies have shown associations between homocysteine and hip fracture risk, perhaps explained by greater bone resorption, and it has been postulated that nutritional intervention with folate or B vitamins has potential to reduce fracture risk.^{13–15} The reduction in OR for hip fracture from 2.6 in the base model to 1.8 after adding homocysteine suggests that effects of renal function on homocysteine may mediate some portion of the association between cystatin-C and hip fracture. Alternatively, homocysteine levels could simply rise as renal function declines without direct involvement in the physiological pathway leading to hip fracture.

Cystatin-C has been shown to correlate highly with direct measures of GFR such as [¹²⁵I]iothalamate clearance, even more so than creatinine-based $eGFR$.²³ Touted advantages of this biomarker include its precision, low inter-individual variability, and independence from muscle mass and body weight,²⁴ although some studies, including the current one, show correlations between cystatin-C and body mass.²⁵ Some evidence was found of an interaction between cystatin-C and BMI, indicating a stronger association between renal function and hip fracture risk in overweight and obese women. This may simply reflect the comparison with overweight women with normal renal function who have half the risk of hip fracture of thinner women with normal renal function. Cystatin-C levels may be superior in measuring mild renal insufficiency. Although the present findings did not refute a linear association between cystatin-C levels and hip fracture, the categorical

data show no association for $eGFR_{cys-c}$ levels greater than 60 mL/min per 1.73 m².

The WHI-OS is a large, diverse cohort of postmenopausal women, allowing the largest investigation to date on this topic to be conducted. Strengths of this study include adjustment for numerous potential confounders, elimination of confounding by current hormone use, evaluation of cystatin-C as a continuous biomarker and in $eGFR_{cys-c}$ categories, and exploration of numerous potential underlying mechanisms. The present study was limited by having a single measurement of cystatin-C and no measurements of bone density, serum calcium, parathyroid hormone, bone-specific alkaline phosphatase, serum creatinine, inflammatory biomarkers, or proteinuria. Too few women had Stage 4 to 5 CKD ($eGFR_{cys-c} < 30$ mL/min per 1.73 m²) to estimate hip fracture risks associated with severe disease. Because there was no criterion standard measure of GFR, the possibility of a direct mechanism linking cystatin-C with hip fracture risk, unrelated to GFR, cannot be excluded. Only 20 hip fractures occurred in minority women, and therefore it was not possible to determine whether differences exist between racial and ethnic groups.

Cystatin-C $eGFR$ levels less than 60 mL/min per 1.73 m² are a strong, independent risk factor for hip fracture in postmenopausal women. Women with low bone density, normal parathyroid hormone and alkaline phosphatase, and Stage 1 to 3 CKD can reduce their fracture risk with treatment.^{26,27} Postmenopausal women with CKD Stage 3 or higher should be considered at high risk and evaluated for bone disease.

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